

Ecological diversification associated with the benthic-to-pelagic transition by North American minnows

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Abstract

Ecological opportunity is often regarded as a key factor that explains why diversity is unevenly distributed across life. Colonization of novel environments or adaptive zones may promote diversification. North American minnows exhibit an ancestral benthic-to-pelagic habitat shift that coincided with a burst in diversification. Here, we evaluate the phenotypic and ecological implications of this habitat shift by assessing craniofacial and dietary traits among 34 species and testing for morphology–diet covariation, convergence and adaptive optima. There were several instances of morphology–diet covariation such as correlations between mouth angle and the consumption of terrestrial insects and between relative gut length and the consumption of algae. After accounting for size and phylogenetic nonindependence, benthic species had longer heads, longer snouts, eyes positioned higher on their head, smaller mouth angles and longer digestive tracts than pelagic minnows. Benthic minnows also consumed more algae but less terrestrial insects, by volume, than pelagic minnows. Lastly, there were three distinct evolutionary regimes and more convergence in morphology and dietary characteristics than expected under a Brownian motion model of evolution. These findings indicate that colonization of the pelagic zone by minnows involved myriad phenotypic and dietary changes associated with exploitation of terrestrial subsidies. Thus, minnows exhibit phenotype–dietary covariation, an expansion of ecological roles and a burst in diversification rates in response to the ecological opportunity afforded by the colonization of a novel habitat.

Introduction

Ecological opportunity resulting from the colonization of novel environments or adaptive zones may promote diversification and explain why diversity is unevenly distributed across life. Many groups exhibit explosive diversification after colonization events, such as African cichlid fishes and Caribbean *Anolis* lizards following the colonization of lakes and islands, respectively (Sturmbauer, 1998; Seehausen, 2006; Pinto *et al.*, 2008; Losos, 2010; Mahler *et al.*, 2010). Such colonization events are thought to provide access to new adaptive zones,

relaxed selection on ecologically important traits and relaxed competitive pressures (Losos & de Queiroz, 1997; Schluter, 2000; Yoder *et al.*, 2010), thus altering the adaptive landscape. Habitat shifts may provide similar possibilities albeit on a smaller scale. For example, many groups of fishes including cichlids, stickleback, char and minnows (among others) have diversified in association with transitions between benthic and pelagic habitats (Rundle *et al.*, 2000; Jonsson & Jonsson, 2001; Ostbye *et al.*, 2006; Cooper *et al.*, 2010; Hulsey *et al.*, 2013). Freshwater glacier and crater lakes, in particular, appear to have provided ample opportunity for diversification along the benthic–pelagic axis. Vast depths, heterogeneous environmental conditions and the ecological opportunity associated with the colonization of novel habitats, in combination, have likely contributed to the ubiquity of this pattern.

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Cyprinid fishes have diversified throughout stream environments across North America after invading from Europe and/or Asia (Imoto *et al.*, 2013; Stout *et al.*, 2016). In North America, the vast majority of these species (i.e. >95%) form a strongly supported clade including approximately 200 species (Hollingsworth *et al.*, 2013), which are united by the osteological character of a small opening at the base of the skull known as the open posterior myodome (OPM). Minnow assemblages are generally not monophyletic such that members of multiple major clades are represented in any particular stream. This is likely due to dramatically changing basin configurations and their impact on fish phylogeny (Mayden, 1998). For example, ancient rivers such as White's River and the Appalachian River may have served as the sites of early minnow diversification and assemblage formation during periods of rapid sea level fluctuations during the Pleistocene (Nagle & Simons, 2012). Minnow assemblages exhibit diverse morphologies, trophic ecologies and habitat preferences (Burrell *et al.*, 2016a,b). An ancestral benthic-to-pelagic

habitat shift that occurred around the Eocene–Oligocene border is hypothesized to have coincided with a burst in minnow species diversification (Hollingsworth *et al.*, 2013). This habitat shift may explain extant minnow species diversity; however, it is unknown what effect this event had on extant minnow ecological diversity.

Here, we evaluate trophic-related phenotypic and dietary diversification of OPM minnows. Hollingsworth *et al.* (2013) hypothesized that a benthic-to-pelagic habitat shift coincided with their proliferation (Fig. 1). To evaluate phenotypic and dietary diversification associated with the benthic-to-pelagic habitat shift, and potential adaptive nature of the North American minnow radiation, we used five successive steps: we (i) accessed and visualized the burst in species diversification and ancestral habitat shift (originally hypothesized by Hollingsworth *et al.*, 2013), (ii) modelled the covariation among phenotypic and dietary variables, (iii) compared these variables between benthic and pelagic minnows, (iv) estimate the direction and magnitude of

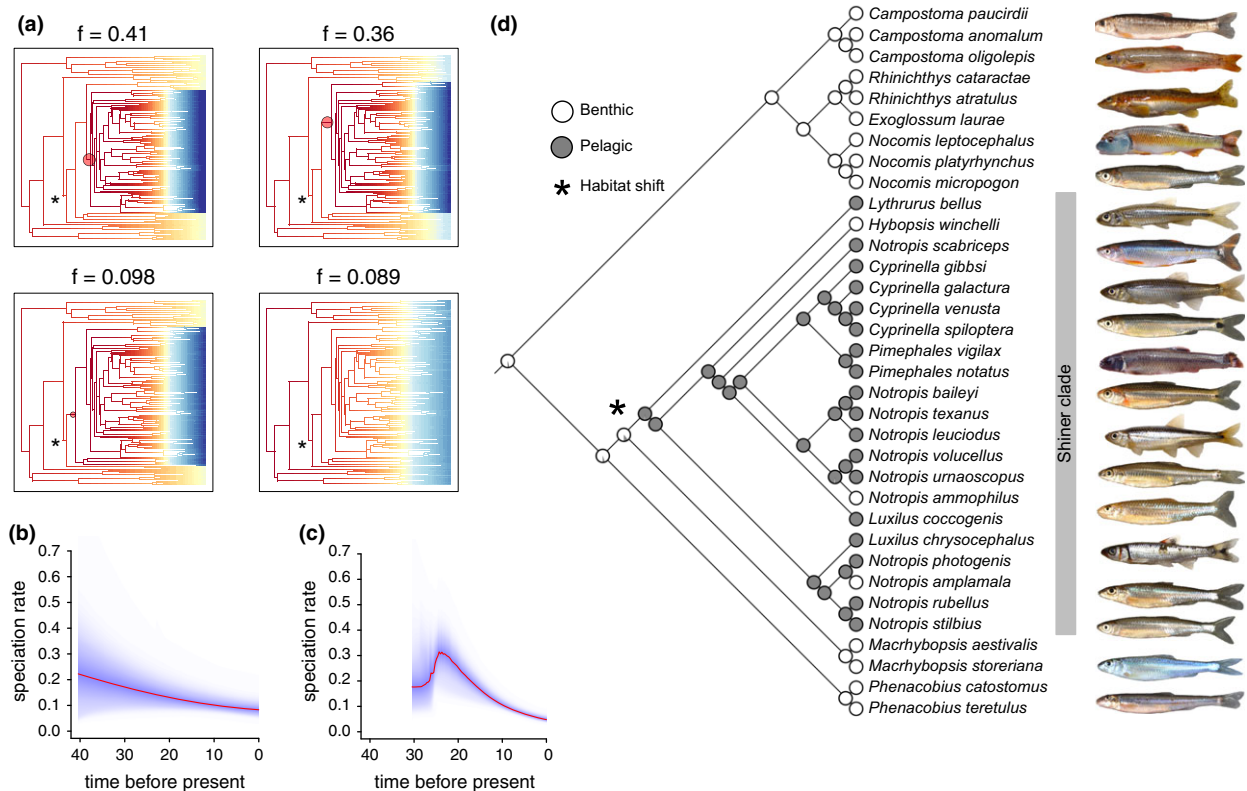


Fig. 1 Visual summary of burst in diversification rate among OPM minnows (a; 95% credible shift sets). Warm colours such as red depict relatively fast rates, and cool colours such as blue depict relatively slow rates. Dots depict the location of discrete rate shifts. Large dots correspond to more probable shifts. Rate-through-time plots depicting the rate of diversification among nonshiners (b) and the shiner clade (c). The ancestral state reconstruction depicts the benthic-to-pelagic habitat shift among OPM minnows (d). The ancestral state reconstruction is pruned to include the species analysed in this study, whereas the credible shift sets (a) and RTT (b, c) analyses are based on the unpruned phylogeny (see Hollingsworth *et al.*, 2013 for ancestral state reconstruction of the unpruned phylogeny). Asterisk denotes the location of the habitat shift.

phenotypic and dietary changes associated with the habitat shift and (v) estimated the evolutionary history of ecological roles by testing for convergent (or nonconvergent) evolutionary regimes (i.e. lineages evolving towards a trait optimum) and associated adaptive optima across the OPM minnow phylogeny.

Materials and methods

Diversification rate analyses

We evaluated the tempo of diversification, particularly the hypothesized burst in diversification rate among OPM minnows originally described by Hollingsworth *et al.* (2013), using BAMM and BAMMtools (Rabosky *et al.*, 2014). BAMM allows for estimation of diversification rates that vary across time and across clades on a time-calibrated phylogeny. We used the maximum clade credibility (MCC) phylogeny originally from Hollingsworth *et al.* (2013). Sequences of *Cytb* and *Rag1* were manually aligned and concatenated, and the phylogeny was estimated using BEAST v1.7.1 (Drummond *et al.*, 2012). Models were run using a birth–death speciation prior, and trees were generated using RAxML v7.04 (Stamatakis *et al.*, 2005). An uncorrelated lognormal molecular clock model was used to temporally calibrate the concatenated phylogeny (see Hollingsworth *et al.*, 2013 for further details). Sequence data and the tree (originally from Hollingsworth *et al.*, 2013) are available on GenBank (KC763652–KC763776) and TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S15034>), respectively. Priors for data analysis in the BAMM input file were set using priors given from the `setBAMMpriors` command in BAMMtools. To account for nonrandom incomplete taxon sampling and the influence it may have on estimation of diversification rates, we specified clade-specific sampling probabilities. Analyses were run for 50M generations, generations were written every 20 000 generations, and event data were written every 10 000 generations. We then visualized changes in diversification rates across the phylogeny and through time using 95% credible shift sets and rate-through-time (RTT) plots. This procedure differs from that used by Hollingsworth *et al.* (2013) in two important ways. First, BAMM utilizes a Bayesian framework; second, we incorporated uncertainty associated with incomplete taxon sampling; and third, variation in diversification rates are inferred without *a priori* selection of a focal clade.

Morphological and dietary measurements

We surveyed the morphology and diets of 174 individuals representing 34 species (Table S1). These species are distributed across the minnow phylogeny such that all major eastern North American lineages are included. Additionally, these species are representative of the

ecological diversity among North American minnows. We quantified craniofacial shape using five linear measurements that describe dimensions of the head and jaws (adapted from Armbruster, 2012) that are known correlates of ecology (López-Fernández *et al.*, 2013). Linear measurements were made to the nearest 0.1 mm with digital callipers. Measurements included standard length (SL), head length (HL), eye diameter (ED), head depth (HD), snout length (SnL) and eye position (EP; see Table S2 for detailed descriptions of measurements). We measured two additional features because of their known association with specialization along the benthic-to-pelagic axis: relative gut length (RGL, Burress *et al.*, 2016a) and mouth angle (MA; Winemiller *et al.*, 1995). The entire digestive tract was dissected and measured from the oesophagus to the anus. Mouth angle was measured using a protractor such that 90° corresponded with a terminal mouth that is parallel with the body (i.e. >90° angles represent superior-oriented mouths, whereas <90° angles represent inferior mouths). The angle was calculated based on the slope of the upper jaw. Only adult specimens were measured to reduce the biases introduced by allometry. All specimens are accessioned in the Auburn University Museum of Natural History (Table S1).

To quantify minnow diets, we analysed the gut contents of 807 individuals representing 34 species. We opportunistically sampled fishes from five streams using a combination of seine and electroshocking: the New and Watauga rivers (Ohio and Tennessee River basins, respectively) in western North Carolina and Halawakee (Chattahoochee River Basin), Uphapee and Hillabee creeks (Tallapoosa River Basin) in eastern Alabama. After collection, the entire digestive tract was dissected and preserved in 10% formalin. Contents from the oesophagus to the posterior end of the anterior bulb (i.e. the foregut) were removed (Sterners & George, 2000; Burress *et al.*, 2016b). Items were examined under a microscope and sorted into eight generalized categories: aquatic insects, terrestrial insects, molluscs, crustaceans, algae, plant material, seeds and sediment. We then estimated the volumetric proportions of each category by spreading the contents on slides and comparing their area to that of material of known volume (Winemiller, 1990).

Ancestral habitat shift reconstruction

To determine whether the 34 species we used to assess morphology and diet were sufficient to characterize the ancestral benthic-to-pelagic habitat shift, we estimated the evolution of habitat use (benthic vs. pelagic) using maximum-likelihood (mk1 model) character state reconstruction in Mesquite v2.75 (Maddison & Maddison, 2011). In this procedure, maximum-likelihood analyses find the ancestral states (e.g. internal nodes) that maximize the probability that the observed

character states (e.g. terminal nodes) would evolve under a stochastic model of evolution (Schluter *et al.*, 1997; Pagel, 1999). The *mk1* model (Lewis, 2001) assumes that any change (i.e. benthic to pelagic or pelagic to benthic) is equally probable. Habitat designations follow Hollingsworth *et al.* (2013).

Trait covariation

To evaluate covariation among variables, particularly between morphological and dietary variables, we used partial least squares canonical analysis (PLS-CA) to model these relationships (Rohlf & Corti, 2000). To account for body size, we calculated phylogenetically size-corrected residuals. Linear measurements were log-transformed, and then, residuals were calculated using the regression with log-transformed SL using the function *phyl.resid* in the R package *phytools* (Revell, 2009, 2012). We used the MCC phylogeny from Hollingsworth *et al.* (2013), pruned to include only the 34 species for which measurements were taken for phylogenetic size correction. RGL, MA, the five craniofacial measurements and eight diet variables were coded as two blocks of variables (e.g. morphological and dietary) and analysed using the *plsca* function in the R package *plsdepot* (Sanchez & Sanchez, 2012). Partial least squares canonical analysis simultaneously assesses correlations among multiple continuous variables from two discrete blocks and thus allows easy interpretation of relationships among two types of variables. The *plsca* function treats the blocks of variables as symmetric such that neither are predictors/responses and maximizes the correlation among variables rather than covariation. To evaluate the importance of habitat, we then compared the five craniofacial measurements, RGL, and MA, and five major dietary items (i.e. three prey categories with many zeros were excluded) between benthic and pelagic species using the *t.test* function in R.

Trait diversification

To describe major axes of craniofacial shape, we performed phylogenetic principle component analysis (function *phyl.pca* [*pPC*] in R package *phytools*; Revell, 2009, 2012) using the phylogenetically size-corrected residuals. We used the MCC phylogeny from Hollingsworth *et al.* (2013) during the *pPCA* procedure, pruned to include only the 34 species from which morphological measurements were taken. Major axes of dietary variation were explored in the same way using the volumetric proportions (%) of eight prey categories derived from gut content analyses. To characterize phylomorphospace and phylotrophospace, we then mapped the phylogeny onto our morphometric and dietary *pPC* scores using *Mesquite v2.75* (Maddison & Maddison, 2011). The values of internal nodes were calculated using weighted squared-change parsimony (Maddison, 1991; Revell *et al.*, 2007).

We define phylotrophospace as the rendering of a phylogeny on a plot of dietary characteristics using squared-change parsimony to act as a companion to phylomorphospace as defined by Sidlauskas (2008). The resulting phylomorphospace and phylotrophospace depicts both the magnitude and the direction of change in trait values along each branch. Then, we plotted the habitat use ancestral state reconstruction onto phylomorphospace and phylotrophospace such that trait and habitat changes can be interpreted simultaneously.

Adaptive landscape

Lastly, we tested for trait convergence using *SURFACE* analysis (Ingram & Mahler, 2013). We used six variables for the model: craniofacial *pPC1-2*, dietary *pPC1-2*, RGL and MA. *SURFACE* analysis uses stepwise Akaike information criterion (AIC) to locate regime shifts on the phylogeny, and then identify whether these shifts are towards convergent regimes. This process involves iteratively adding regime shifts (*k*), then iteratively removing shifts to identify convergent regimes (*k'*) during successive forward and backward phases of the analysis using an Ornstein–Uhlenbeck model of evolution. Regimes are added and subsequently collapsed into convergent regimes based on AIC scores. The reduction in model complexity (*k-k'*) corresponds to the number of regimes that can be collapsed into an existing regime (i.e. convergence; Δk). These evolutionary regimes were visualized by overlaying the convergent and nonconvergent regimes onto the phylogeny. Lastly, to determine whether convergence was significantly greater than could result from a null expectation (i.e. Brownian motion), we generated 100 simulated data sets consisting of six variables under a 'null' model using the *surfaceSimulate* function (Ingram & Mahler, 2013). We then calculated the probability of the observed data (i.e. the degree of convergence; Δk) based on the null distribution.

Results

Diversification and habitat shift reconstruction

Diversification rate analysis indicates one discrete rate shift near the origin of the shiner clade (Fig. 1a). In different shift configurations, the location of the shift varies in its inclusion or exclusion of *Pteronotropis*, the group that has a basal relationship with the remaining members of the shiner clade (Fig. 1a). Rate-through-time analyses also indicate that diversification rates have slowed through time for all nonshiner clades (Fig. 1b), but increased abruptly approximately 25 Mya within the shiner clade (Fig. 1c). Ancestral state reconstruction reveals a discrete shift from benthic-to-pelagic habitat that also corresponds with the origin of the shiner clade (Fig. 1d).

Trait covariation

Partial least squares canonical analysis of five size-corrected linear morphological measurements and the volumetric proportions of eight generalized prey items revealed several morphology–diet correlations (Fig. 2). Craniofacial measurements (e.g. SnL, HD, HL and EP) were positively correlated with the consumption of crustaceans and molluscs and negatively correlated with the consumption of aquatic insects (Fig. 2). RGL was positively correlated with the consumption of vegetation and algae and negatively correlated with consumption of terrestrial insects and seeds (Fig. 2). ED was positively correlated with consumption of aquatic and terrestrial insects (Fig. 2). Additionally, MA was positively correlated with consumption of terrestrial insects and seeds and negatively correlated with consumption of sediment and vegetation (Fig. 2). Some craniofacial measurements and dietary variables also differed between benthic and pelagic species (Fig. 3). HD and ED were not significantly different between benthic and pelagic species (Fig. 3a); however, benthic species had significantly longer HL, SnL, RGL and higher EP than pelagic species (Fig. 3a). Additionally, pelagic species had significantly larger mouth angles (Fig. 3a). Benthic species consumed more algae, by volume, than

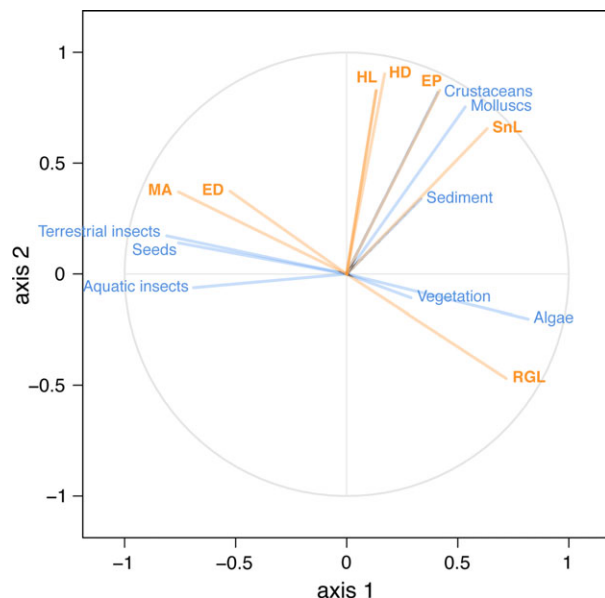


Fig. 2 Partial least squares canonical analysis depicting the covariation between dietary (blue) and morphological variables (orange): body depth (BD), head length (HL), snout length (SnL), eye position (EP), eye diameter (ED), mouth angle (MA) and relative gut length (RGL). Variables near the edge of the circle are better represented by the model. Variables near one another are positively correlated, whereas variables opposite one another are negatively correlated. Uncorrelated variables are orthogonal. All measurements, except MA, are phylogenetically size-corrected residuals.

pelagic species ($T = 2.68$; $P = 0.019$; Fig. 3b), but did not consume different proportions of vegetation ($T = 1.25$; $P = 0.23$), sediment ($T = 0.231$; $P = 0.819$) or aquatic invertebrates ($T = 1.14$; $P = 0.273$), than pelagic species (Fig. 3b). Pelagic species consumed more terrestrial insects, by volume, than benthic species ($T = 4.99$; $P < 0.0001$; Fig. 3b).

Trait diversification

Phylogenetic principal component analysis of five size-corrected linear measurements revealed major axes of craniofacial shape variation among minnows. pPC1 explained 93.4% of the variation in craniofacial shape. Positive pPC1 scores were associated with a small head, whereas negative scores were associated with a robust head (Table S5; Fig. 4a). pPC2 explained 3.7% of the variation in body shape and represents variation in eye diameter, snout length and head length (Table S5; Fig. 4a). Benthic minnows had more variable craniofacial morphologies, particularly in the robustness of the head, length of the head and length of the snout (Fig. 4a). Major shape changes across the phylogeny were associated with *Campostoma* (i.e. elongation of the head and snout) and *Nocomis* (i.e. increase in robustness of the head; Fig. 4b). Morphological changes associated with the benthic-to-pelagic habitat shift include reduction in head size and more mesial eye position (Fig. 4b).

Phylogenetic principal component analysis of minnow gut contents revealed major axes of dietary variation (Fig. 4c). pPC1 explained 87.5% of the variation in diet (Table S6). Positive pPC1 scores were associated with algae, whereas negative pPC1 scores were associated with aquatic insects (Table S6). pPC2 explained 5.8% of the variation in diet and represented variation in the consumption of terrestrial insects and seeds (Table S6). Benthic minnows exhibited more variable diets than pelagic minnows, particularly in their degree of omnivory (Fig. 4c). Major dietary changes across the phylogeny were associated with *Campostoma* and *Nocomis*, which reflects the evolution of algivory (Fig. 4d). Dietary changes associated with the benthic-to-pelagic habitat shift were higher degrees of carnivory and increased consumption of terrestrial insects (Fig. 4d).

Minnow species also varied in their RGL, which ranged from 3.0 to 3.3 in *Campostoma* to 0.65–0.69 in some *Notropis* and *Cyprinella* (Table S3). Minnows also exhibited variable mouth angles that ranged from subterminal to superior in orientation and that varied from 71 to 78° in *Campostoma*, *Phenacobius* and *Macrhybopsis* to 110–120° in *Lythrurus*, *Cyprinella* and some *Notropis* (Table S3). Benthic minnows exhibited less variable mouth angles, which tended to be inferiorly oriented, but more variable RGL than their pelagic counterparts (Fig. 4e). Major changes across the phylogeny included elongation of the digestive tract in *Campostoma* and *Nocomis* (Fig. 4f). The benthic-to-pelagic shift

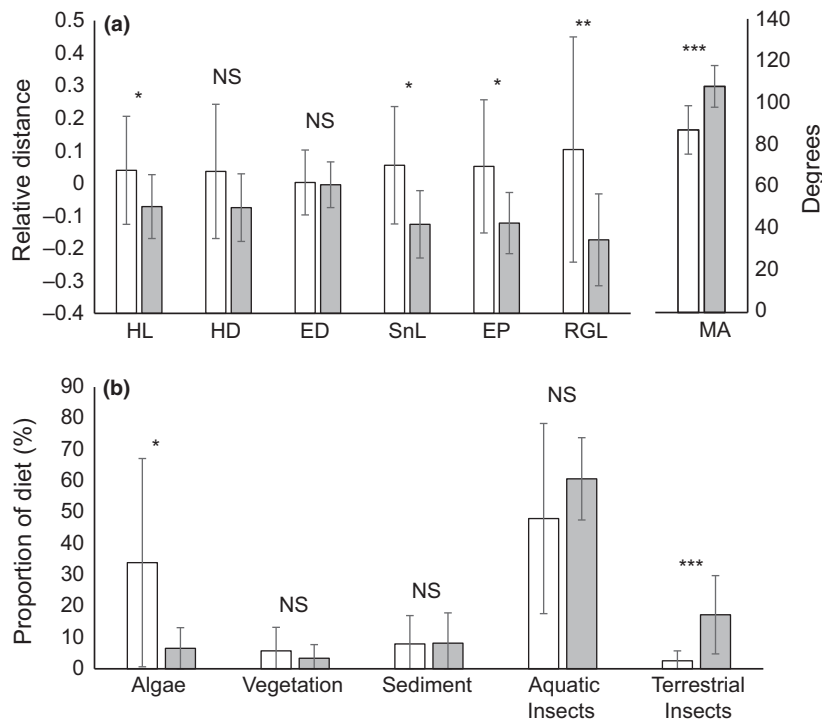


Fig. 3 Mean (\pm SD) measurements of benthic (white bars) and pelagic minnows (grey bars; a): head length (HL), head depth (HD), eye diameter (ED), snout length (SnL), eye position (EP), relative gut length (RGL) and mouth angle (MA). Mean (\pm SD) proportion of generalized prey items of benthic and pelagic minnows (b). * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$.

corresponded with reduced RGL and more superiorly positioned mouths (Fig. 4f).

Adaptive landscape

SURFACE analysis resulted in seven shifts in evolutionary regime, and after collapsing convergent regimes, there were three distinct evolutionary regimes: two convergent and one nonconvergent (Table 1; Fig. 5a). This degree of convergence ($\Delta k = 4$) was not sampled in the null distribution ($\Delta k = 1-2$) and thus was more than expected from a Brownian motion model of evolution (Table 1; Fig. 5b). The adaptive optima associated with each distinct evolutionary regime were characterized by 1) robust head, inferior MA, long RGL and consumption of algae (e.g. *Campostoma* and some *Nocomis*), 2) small head, inferior to terminal MA, intermediate RGL and consumption of aquatic insects (e.g. *Rhinichthys*, *Cypripinella* and *Pimephales*) and 3) small head, terminal to superior MA, short RGL and consumption of terrestrial and aquatic insects (e.g. *Notropis*; Fig. 5c,d,e).

Discussion

Ecological diversification along the benthic–pelagic axis

Our analyses corroborate those of Hollingsworth *et al.* (2013) that an ancestral benthic-to-pelagic habitat shift coincided with or immediately preceded a burst in diversification rates among minnows (Fig. 1). We further

demonstrate that the benthic-to-pelagic habitat shift coincided with several morphological modifications: inferior to superior orientation of the jaws, shortening of the digestive tract, head length, and snout length, and more mesial position of the eyes. These changes in craniofacial morphology were also associated with dietary differences such as consumption of lower proportions of algae and higher proportions of terrestrial insects. Long heads and snouts and eyes positioned high on their head are generally associated with species that feed from the benthos and sometimes plunge their heads into the substrate (Winemiller *et al.*, 1995). Thus, this morphology likely facilitates benthic minnows, such as *Nocomis* and *Campostoma* that forage for algae and invertebrates along the substrate. Interestingly, benthic and pelagic minnows did not differ in their relative exploitation of aquatic insects. This is likely due to benthic species feeding directly from the substrate, whereas pelagic minnows may feed on the same items but via drift (Grossman *et al.*, 1982). Feeding via drift is likely facilitated by the variable flow regimes associated with stream environments that frequently disrupt benthic invertebrate communities (Bunn & Arthington, 2002) and transport terrestrial subsidies into the stream (Junk *et al.*, 1989). The exploitation of terrestrial subsidies, via a combination of from the water column and water surface, may be facilitated by these species having superiorly oriented mouths (Burrress *et al.*, 2016b). Likewise, the inferiorly oriented mouths of benthic minnows may preclude those species from effectively consuming prey near or at the water surface (Gerking, 1994).

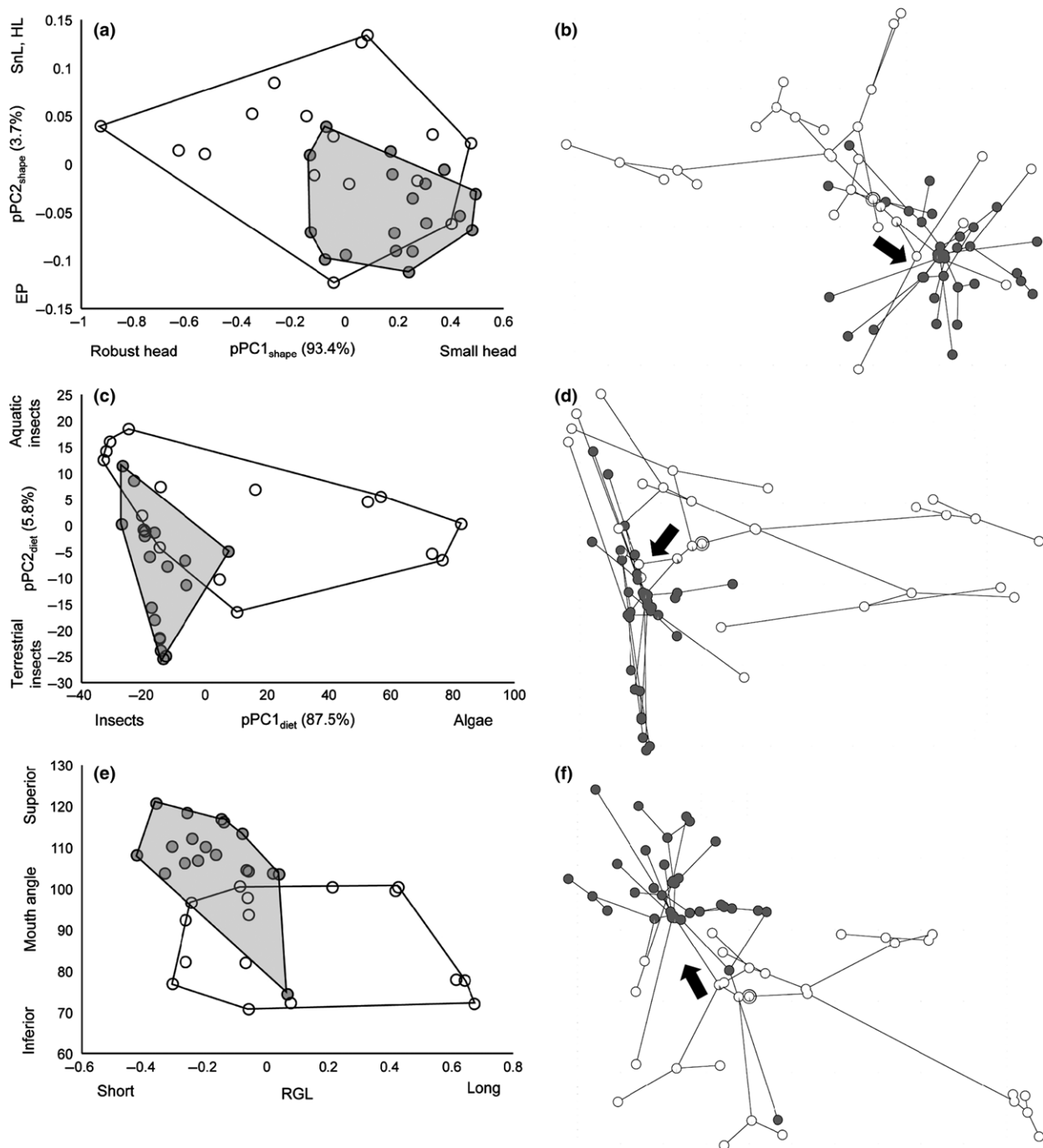


Fig. 4 Phylogeny- and size-corrected ecological relationships among minnows: craniofacial morphospace (a) and phylomorphospace (b), trophospace (c) and phylotrophospace (d), relative gut length (RGL) and mouth angle (MA; e), and RGL and MA with overlaid phylogeny (f). Colours of terminal dots correspond to benthic (white circles) and pelagic (grey circles) minnows based on the designations from Hollingsworth *et al.* (2013). Text describes the trait variation explained by each axis (see Tables S5 and S6 for factor loadings). Internal nodes of overlaid phylogenies were estimated using squared-change parsimony (Pagel, 1999; Schluter *et al.*, 1997). Pie diagrams on internal nodes depict the likelihood of each habitat state (benthic or pelagic). Branches depict both the direction and magnitude of shape change. Arrows emphasize the direction of change along the branch associated with the benthic–pelagic habitat shift.

Table 1 Summary of regime shifts and convergence in craniofacial shape, diet, relative gut length and mouth angle from SURFACE analysis. Values are from the best supported Hansen model. The null distribution is based on 100 simulated data sets (values represent the mean and range) based on a Brownian motion model of evolution. Statistics indicate the # of regime shifts (k), number of distinct regimes (k'), the reduction in complexity when accounting for convergence (Δk), the # of shifts towards convergent regimes occupied by multiple lineages (c), the # of convergent regimes reached by multiple lineages (k'_{conv}) and the proportion of shifts that are towards convergent regimes (c/k).

Model	k	k'	Δk	c	k'_{conv}	c/k
Observed	7	3	4	6	2	0.86
Null	3.0 (2–5)	2.8 (2–5)	0.3 (0–2)	0.6 (0–4)	0.3 (0–2)	0.18

Benthic and pelagic minnows exhibited more differences in their craniofacial morphologies than in their diets (Fig. 3). This suggests that the morphological differences may relate to where and how minnows forage rather than what they consume. Additionally, dietary patterns are labile and many fishes are generally opportunistic (Gerking, 1994). For example, some pelagic minnows consume fractions of vegetation and detritus (i.e. *Cyprinella*, and *Pimephales*), thereby creating some dietary overlap with benthic minnows; however, these items may also have been consumed via drift rather than directly from the substrate. Regardless, these species highlight the continuous nature of the benthic–pelagic axis, such that species may specialize along the axis and are not necessarily discretely benthic or pelagic (Kusche *et al.*, 2014). These species may be generalists or have little affinity to specific habitats. These species do not have the modifications for benthic existence like robust heads, long snouts or long digestive tracts, but may still live close to the substrate and therefore are more likely to consume algae or aquatic insects and detritus (Burress *et al.*, 2016b).

Pelagic minnows occupy a smaller volume of morphospace and trophospace (Fig. 5a,b) and also exhibit less variation in specific morphological measurements and dietary variables (Fig. 3), than their benthic counterparts. Thus, the benthic-to-pelagic shift may have been somewhat constrained such that ecological roles of pelagic minnows are more specialized than those of their benthic counterparts. Indeed, benthic minnows appear to form two discrete guilds: herbivores and carnivores, whereas pelagic minnows are strictly carnivorous. Physiological constraints may also contribute to this pattern. For example, short digestive tracts exhibited by pelagic minnows likely preclude them from efficiently digesting plant materials, thereby restricting them to carnivorous diets (German *et al.*, 2010). In contrast, herbivorous or highly omnivorous minnows may preferentially assimilate invertebrates despite physiological and mechanical adaptations for digesting cellulose-rich foods (Burress *et al.*, 2016b). This disparity in

physiological constraints results in benthic minnows having more dietary flexibility than pelagic minnows. This may be particularly useful when environmental conditions are unstable and resource availability is unpredictable (Junk *et al.*, 1989; Winemiller, 1990).

Adaptive radiation?

Explosive diversification, the rapid accumulation of lineages and adaptive radiation (i.e. the rise of ecological diversity and its corresponding adaptations) are conceptually different despite their frequent conflation (Givnish, 2015). Many contemporary definitions of adaptive radiation emphasize the importance of rates, specifically bursts in speciation and/or phenotypic diversification (Glor, 2010); however, this may not reconcile with how the term was originally used such that adaptive radiations could occur gradually (Simpson, 1953). Hollingsworth *et al.* (2013) characterized OPM minnows as an explosive radiation, yet described an ancestral habitat shift that coincided with a burst in their diversification and thus a potential adaptive component. Here, we demonstrate that this transition from benthic-to-pelagic habitats included an expansion of minnow phenotypic and trophic diversity. Specifically, shorter heads and snouts, shorter digestive tracts and more superiorly oriented mouths facilitated the exploitation of terrestrial subsidies. Thus, OPM minnows appear to satisfy the criteria of an adaptive radiation (Glor, 2010; Givnish, 2015) such that the evolution of novel ecologies (e.g. diets) corresponds with the evolution of novel adaptations (e.g. phenotypes) and the exploration of novel habitats (e.g. the pelagic zone).

Glacier, crater and rift valley lakes are common sites of adaptive radiation among fishes such as stickleback, white fish and cichlids (Schluter, 1993; Rundle *et al.*, 2000; Ostbye *et al.*, 2006; Seehausen, 2006). Lakes provide relatively stable environmental conditions and a discrete environment such that adaptive radiations proceeded within the lake and thus in some degree of sympatry (Schliewen *et al.*, 1994). These factors facilitate the importance of disruptive selection and the development of accommodative processes like resource partitioning to mitigate resource-based competition, potentially drive assortative mating, and subsequent diversification (Kocher, 2004). Minnows provide several contrasts with existing textbook examples of adaptive radiation among fishes. Firstly, the minnow adaptive radiation occurred in streams rather than lakes, yet utilized a common environmental gradient, the benthic-to-pelagic axis, as the ecological basis of diversification. The benthic-to-pelagic axis is physically constrained in streams relative to lakes due to the generally shallower habitat. However, evolutionary transitions towards a pelagic lifestyle may have facilitated dispersal and subsequent geographic isolation and diversification via allopatric mechanisms. Alternatively,

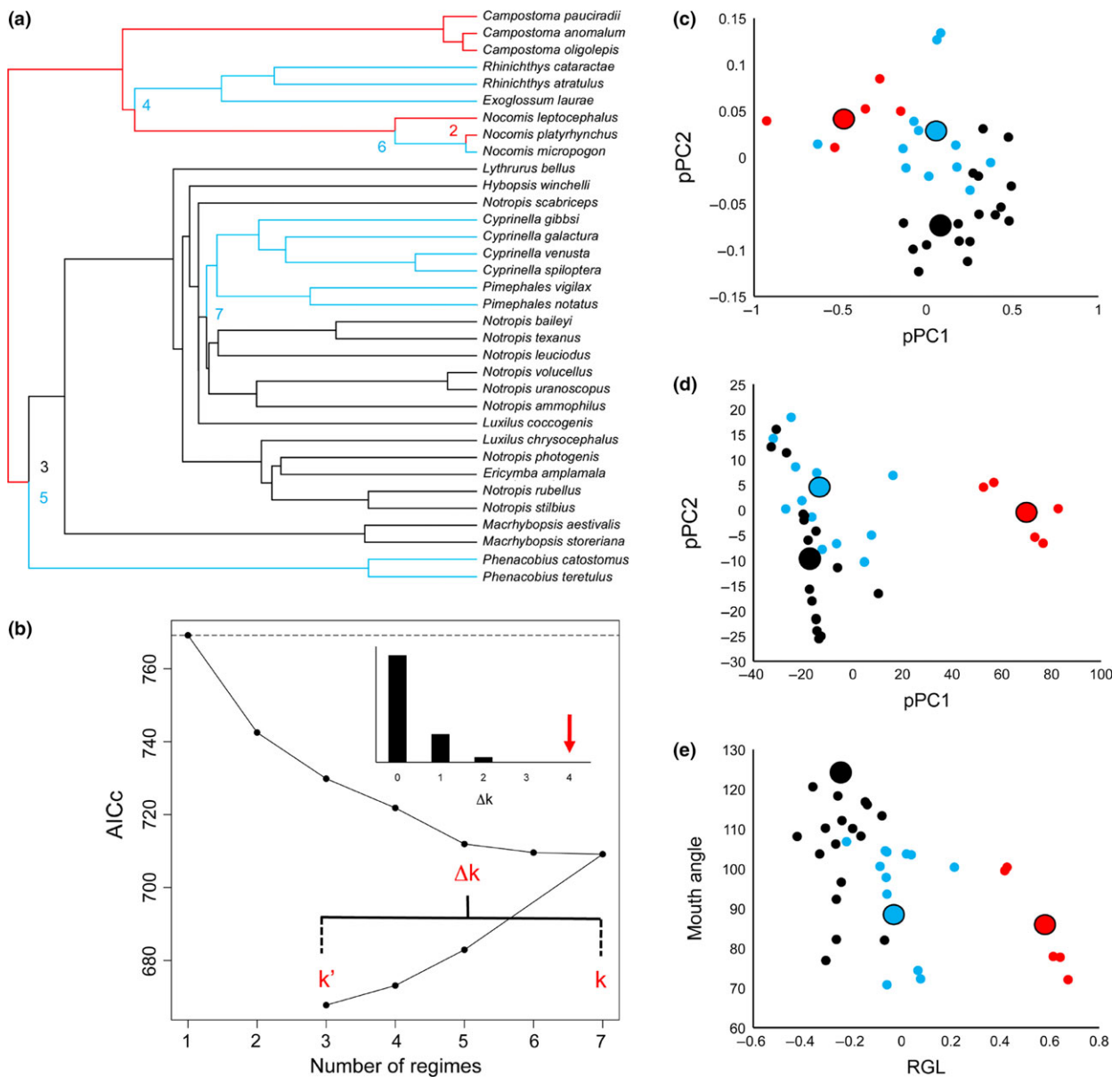


Fig. 5 Convergent (coloured) and nonconvergent (grey scale) evolutionary regimes across the OPM minnow phylogeny (a). Numbers indicate the order in which the regimes were added during the forward phase of the analysis. Changes in AICc during the forward and backward phases of the analysis (b). The null distribution of Δk values is inset. The red arrow indicates the position of the observed data. The adaptive landscape based on (c) craniofacial morphology, (d) diet and (e) mouth angle and relative gut length (RGL). Large dots represent trait optima, and small dots represent the observed trait values. Colour codes are consistent across panels.

unstable flow regimes that constantly disturb benthic habitats (i.e. stream channel morphology) may also select for pelagic foraging, particularly during periods of early basin formation. Indeed, river basins are young and flow regimes are unstable in south-eastern North America (Nagle & Simons, 2012), where most minnow diversity is concentrated (Boschung & Mayden, 2004), and therefore also impose environmental conditions in which constant or prolonged disruptive selection due to

competition-based mechanisms was an unlikely source of initial diversification among minnows. This would corroborate a prevailing hypothesis that stabilizing processes such as resource partitioning to reduce competition are unnecessary due to environmental instability (i.e. stochastic theory; Grossman *et al.*, 1982). Indeed, despite the existence of several ecological guilds, many minnows, particularly the pelagic species, exhibit significant phenotypic and dietary overlap. Although, after

the abrupt increase in diversification following the benthic-to-pelagic habitat shift, these rates slowed through time (Fig. 1; Hollingsworth *et al.*, 2013). This pattern of diversification provides evidence for an ecological constraint (Losos, 2011). Perhaps after pelagic minnows became diverse, there were limiting factors such as competition for food or space as evidenced by the phenotypic and dietary overlap among numerous species (Schoener, 1974; Connell, 1980).

Colonization events are thought to provide access to new adaptive zones, relax selection on ecologically important traits and relax competitive pressures (Losos & de Queiroz, 1997; Schluter, 2000; Yoder *et al.*, 2010). The relative importance of these factors during the minnow habitat shift is unclear. Presumably, pelagic minnows could have experienced competitive release from existing benthic minnows as well as other benthic-oriented groups such as suckers (Catostomidae), sculpin (Cottidae) and darters (e.g. *Etheostoma* and *Percina*) that occupy the same streams (Boschung & Mayden, 2004). Indeed, after the initial burst, diversification rates slowed, suggesting density dependence or ecological constraint (Losos, 2010, 2011). The benthic-to-pelagic habitat shift occurred approximately 30 Mya (Hollingsworth *et al.*, 2013). This timing corresponds to the Eocene–Oligocene border, which was marked by dramatic global cooling as well as trends of lineage extinction and origination (Thomas, 1992; Graham, 1999; Luterbacher *et al.*, 2004). In North American streams, this coincided with the proliferation of darters (Near *et al.*, 2011). Therefore, competition with darters, which are benthic specialists, may have promoted the benthic-to-pelagic habitat shift by minnows. Darters have diversified simultaneously in the same streams as minnows (Near *et al.*, 2011; Hollingsworth *et al.*, 2013), yet have not explored the benthic–pelagic axis. Most darters lack swim bladders (Evans & Page, 2003) and thus are more constrained to benthic lifestyles than minnows. Many radiations of terrestrial insects also originated during the Oligocene (Wilson, 1978) and may have increased food availability in the pelagic zone and thereby further facilitated the benthic-to-pelagic shift in minnows (Cavender, 1991).

Interspecific competition for benthic resources may have driven the colonization of the pelagic zone by minnows; however, the subsequent burst in diversification could be explained by additional mechanisms. For example, the prevalence of nuptial coloration and sexual dichromatism is higher in the more visually dependent pelagic species compared to their benthic counterparts that rely more heavily upon chemical cues while foraging. Sexual selection is known to play a role in diversification (Barracough *et al.*, 1995; Kazancıoğlu *et al.*, 2009) and may have led to an interaction with the ecological opportunity afforded by the colonization of the pelagic zone that drove the burst in diversification among OPM minnows (Wagner *et al.*, 2012).

The diversity of minnows throughout North American streams arose via adaptive radiation. Colonization of the pelagic zone was associated with changes in morphology and diet, expansion of ecological roles and a burst in speciation among minnows. Subsequent slowing of diversification rates signals diversity dependence, likely in response to niche filling (Freckleton & Harvey, 2006). Utilization of the benthic–pelagic habitat axis during adaptive radiation has been ubiquitous among lake-dwelling fishes such as stickleback, white fish and cichlids (Schluter, 1993; Rundle *et al.*, 2000; Ostbye *et al.*, 2006; Seehausen, 2006). The minnow adaptive radiation may be unique such that they have utilized the benthic–pelagic axis during their proliferation throughout stream environments.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Table S1 Material examined for morphological measurements.

Table S2 Description of linear measurements.

Table S3 Morphological measurements (mean per species; mm): standard length (SL), head length (HL), eye diameter (ED), head depth (HD), snout length (SnL), eye position (EP), relative gut length (RGL), and mouth angle (MA).

Table S4 Gut contents (% by volume).

Table S5 Factor loadings for morphological variables. Note that only the first two axes were used in analyses in the paper.

Table S6 Factor loadings for dietary variables. Note that only the first two axes were used in analyses in the paper.

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